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Antirheumatic medication and salivary MMP-8, a biomarker for periodontal disease

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Abstract

Objective: To investigate the impact of antirheumatic medications on salivary matrix metalloproteinase (MMP) -8 levels and MMP-8/TIMP (tissue inhibitor of MMPs)-1 ratio in patients with rheumatoid arthritis (RA) and periodontal findings during a 1-year follow-up.

Materials and Methods: Salivary MMP-8 was measured by an immunofluorometric assay and TIMP-1 by an enzyme-linked immunosorbent assay of 53 patients with early untreated RA (ERA), naïve to synthetic disease modifying antirheumatic drugs (DMARDs), of 28 patients with chronic RA (CRA), candidates for biologic DMARDs and of 43 age- and sex-matched controls. Periodontal health was evaluated by bleeding on probing (BOP), pocket depth (PD), and periodontal inflammatory burden index (PIBI). Examinations were conducted twice for RA patients and once for controls.

Results: Salivary MMP-8 level and MMP-8/TIMP-1 ratio associated positively with PIBI in patients with chronic RA (MMP-8: $p<0.001$ at baseline, $p=0.002$ after follow-up; MMP-8/TIMP-1 ratio $p<0.001$, $p=0.003$, respectively) and in controls (MMP-8: $p=0.010$, MMP-8/TIMP-1 ratio: $p=0.010$). Salivary MMP-8 levels were highest at the early stage of RA. The used DMARDs, synthetic or biologic, did not affect salivary MMP-8 concentrations.

Conclusions: The use of synthetic or biologic DMARDs did not affect salivary MMP-8 levels in RA patients regardless the duration of RA.

Key words: Matrix metalloproteinases, saliva, periodontitis, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) and periodontitis are both inflammatory diseases with progressive chronic nature. Numerous earlier studies have addressed the association and causality of these diseases (Araújo, Melo, & Lima, 2015; Mikuls et al., 2012; Berthelot & Le Goff, 2010; Payne, Golub, Thiele & Mikuls, 2015). *Porphyromonas gingivalis* is reported actually to trigger RA through protein citrullination (Mikuls et al., 2012). This bacterium has been suggested to contribute to the development of RA through the citrullination of arginine residuals by endogenous peptidylarginine deiminase enzyme (Routsias, Goules, Goules, Charalampakis & Pikazis, 2011). Matrix metalloproteinases (MMPs) are a group of genetically separate but structurally related proteinases having a role in normal and pathological tissue regenerating, but also in inflammatory tissue destruction (Nukarinen et al., 2016). They are detected in crevicular and oral fluids and also in synovial fluid related to periodontal and rheumatic inflammation, respectively (Sorsa et al., 2006; Tchetverikov et al., 2004). The plurality and overflow of MMPs may produce pathological tissue loss leading to increased MMPs and TIMPs ratio in saliva/oral fluid (Sorsa et al., 2006) and synovial fluid in inflamed joint (Tchetverikov et al., 2004). MMP-8 is the major collagenolytic MMP affecting gingiva and oral fluids in periodontitis originating mainly from polymorphonuclear neutrophils. MMP-8 can efficiently degrade both type I collagen in the diseased periodontium and type II collagen in the diseased joint cartilage (Sorsa, Uitto, Suomalainen, Vauhkonen & Lindy, 1988; Hasty, Jeffrey, Hibbs & Welgus, 1987). Salivary MMP-8 has been suggested as a biomarker in the monitoring of periodontitis (Sorsa et al., 2010; Nwhator et al., 2014; Heikkinen et al., 2016; Sorsa et al., 2016; Rathnayake, Gieselmann, Heikkinen, Tervahartiala & Sorsa, 2017; Sorsa, Gieselmann, Arweiler & Hernandez, 2017).

Since the 1990s the pharmacotherapy of RA has been revolutionized with early initiation of conventional disease modifying anti-rheumatic drugs (DMARDs) and with the discovery and use of biologic DMARDs (Smolen et al., 2014). Our knowledge about the effect of biological DMARDs in the oral cavity and on salivary MMP-8 is very limited. Use of anti-TNF-α antibody has been reported to have an influence on salivary biomarkers in RA patients [interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) but not MMP-8)] (Mirrielees et al., 2010). With this background, we investigated whether the introduction of DMARDs, synthetic or biologic, has an impact on salivary MMP-8 levels and if the MMP-8 levels and MMP-8/TIMP-1 ratio in saliva associate with periodontal findings in RA under the treatment, either with conventional or biological DMARDs. We previously described that the patients even with early, untreated disease had poor periodontal health (Äyräväinen et al., 2017). Earlier, Monsarrat et al., (2013) reported improved RA disease activity scores among RA patients after periodontal treatment. Thus, the impact of DMARDs on salivary biomarkers and the possibility to use salivary MMP-8 as a biomarker when assessing periodontal disease and its treatment might have practical implications among patients with RA. We hypothesized that the MMP-8 concentrations and MMP-8/TIMP-1 ratio in saliva differ between the various RA patient groups and are modified under treatment with different DMARDs.

Materials and Methods

Study design

We invited RA patients from Department of Rheumatology at the Helsinki University Hospital to participate in this prospective follow-up study. Study groups consisted of 53 untreated early RA (ERA) patients and 28 chronic RA (CRA) patients with inadequate response to synthetic DMARDs. Control subjects (n=43) of same age, gender and from same living area as the RA patients were selected from the national database (Statistics Finland). The original study plan was to collect 50 ERA patients, 50 CRA patients and 50 population controls for both of these patient groups, i.e. a

total of 200 subjects. However, due to the strict inclusion criteria and other practical issues, we could not recruit consecutive patients. Many candidates also refused to participate this study for practical or personal reasons. The population controls were selected to be representative controls for ERA and CRA patients.

The study protocol as well as patients and the control subjects in detail have been earlier described (Äyräväinen et al., 2017). In brief, rheumatological and oral examinations in RA patients were conducted twice, first at baseline and later, after initiation of new DMARD treatments, with a mean follow-up of 15.9±6.1 months. After the first examinations, ERA patients started treatment with synthetic DMARDs consisting of methotrexate (MTX), sulfasalazine (SSZ), hydroxychloroquine (HCQ) and leflunomide (LEF) in various combinations. CRA patients started their first biological DMARDs comprising TNF-α inhibitors or non-TNF-α biologicals mainly combined with ongoing treatment with MTX. Control participants were examined once.

We included RA patients between the ages of 18-70. Rheumatological and dental examinations were conducted by one rheumatologist and one dentist blinded from the oral and clinical conditions of the patients, respectively. The dental examinations were conducted in the Department of Oral and Maxillofacial Diseases. The study participants gave written informed consent to participate in the study. The study protocol had been approved by the independent review board of the Helsinki and Uusimaa Hospital District (no 240/2004, date 16.6.2004), and the study was carried out according to the principles of the Declaration of Helsinki.

Study population

The ERA patients were mostly female (85%) with a mean (± SD) age of 51±15 years. The symptoms of RA had been present for 10.4±17.1 months. The CRA patients also were mostly

female (82%) with a mean age of 52±11 years. They had chronic disease with a mean duration of RA of 176±116.8 months. The patients met the criteria for RA according to the 1987 classification criteria (Arnett et al., 1988). The mean age of the control participants (88% were women) was 56±13 years. During the study 6 ERA patients and 2 CRA patients interrupted the study for personal reasons. One ERA patient died between the dental and rheumatological re-examinations.

Periodontal parameters and inflammatory burden

Dental examination with panoramic jaw tomograms and bite-wing x-rays was recorded of all study participants. The number of teeth examined was 28, since third molars were excluded in the recording (Oral Health Surveys Basic Methods, 1997). In the case of dental problems, the study participants were advised to visit their dentists during the follow-up. Of the ERA patients, 45.7 % reported having periodontal treatment during the follow-up, compared with 44.0% of the CRA patients, respectively. However, we had no detailed data regarding the dental treatments given.

Periodontal parameters bleeding on probing (BOP) and probing depth (PD) (at four sites per every tooth) were recorded (Ainamo & Bay, 1975; Nieminen et al., 1995). Periodontal inflammatory burden index (PIBI) was assessed as described previously (Lindy, Suomalainen, Mäkelä, &Lindy, 2008).

Unstimulated (USFR) and stimulated (SSFR) saliva samples were collected for 5 minutes. Paraffin wax chewing was used for stimulation of saliva secretion. Flow rate was recorded as milliliters per min (Navazesh & Kumar, 2008; Villa, Connell & Abati, 2015).

MMP-8 in saliva

Analysis of MMP-8 levels in saliva was performed as described and reported previously (Hemmilä, Dakubu, Mukkala, Siitari & Lövgren 1984; Hanemaaijer et al., 1997; Mäntylä et al., 2003). The detection limits and inter-assay coefficients of variation were 0.08 ng/mL and 7.1 % for MMP-8 (Rathnayake et al., 2013).

TIMP-1 in saliva

TIMP-1analysis was conducted using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (R&D Systems Europe Ltd, Abingdon, UK). The detection limits and inter-assay coefficients of variation were 7.8 pg/mL and 3.4 % for TIMP-1 (Rautelin et al., 2009).

Rheumatological examinations

The number of swollen and tender joints (66/68 joint count and 28 joint count) were recorded. RA patients gave estimation of disease activity by the patient`s global assessment (PGA) scale (100 mm visual analogue scale). Disease Activity Score (DAS28) was calculated from the number of tender and swollen joints (28-joint count), patient global assessment (PGA) and erythrocyte sedimentation rate (ESR) (Prevoo et al., 1995). Analyses from blood samples were conducted for rheumatoid factor (RF), anticyclic citrullinated peptide antibody (CCPAb), plasma C reactive protein (CRP), and ESR. Response for RA treatment was evaluated according to the European League Against Rheumatism (EULAR) criteria (van Gestel et al., 1996; Fransen & van Riel 2005).

Antirheumatic medication

After the baseline examinations, synthetic DMARDs comprising MTX, SSZ, HCQ and LEF either as monotherapy or in different combinations (mono-, double or triple therapy) were started to ERA patients. Seventeen (37.0 %) of the ERA patients were on monotherapy (mostly MTX, two patients

had LEF), 18 (39.1 %) were on double DMARD therapy (MTX+SSZ, MTX+HCQ, SSZ+HCQ or combinations with LEF) and 9 (19.1 %) were on triple DMARD therapy (MTX+SSZ+HCQ). Furthermore, low-dose (≤10 mg prednisolone equivalent) oral glucocorticoids were used by 28.3 % of the ERA patients.

Before the introduction of biological DMARDs, 8 (28.6 %) of CRA patients were on monotherapy, 11 (39.3 %) on double therapy and 7 (25.0 %) on triple therapy. After the baseline investigations, the CRA patients started biologic DMARDs consisting of TNF-α inhibitors: adalimumab in 9/27 (33.3 %) of patients, etanercept in 17/27 (63.0 %), golimumab in 2/27 (7.4 %) and certolizumab pegol in 1/27 (3.7 %) of patients; or non-TNF-α biologicals: interleukin-1 inhibitor anakinra in 1/27 (3.7 %) of patients, or anti-B-cell antibody rituximab in 2/27 (7.4 %) of patients, mainly combined with MTX. Three CRA patients were on LEF. The biologic DMARDs could be changed if indicated during the follow-up. At follow-up examination, 85.2 % of the CRA patients were on a TNF-α inhibitor and 7.4 % were on a non-TNF-α biological. 74.1 % of the CRA patients used low-dose prednisolone.

Both ERA and CRA patients were treated with intra-articular injections and non-steroidal anti-inflammatory drugs (NSAIDs) to suppress joint swelling and pain.

Statistical methods

The results are given as medians with IQRs (25–75%; non-parametric distribution) or in means with SDs (parametric distribution). Non-parametric Kruskal-Wallis test and Mann-Whitney U-test were used when comparing independent samples, and Wilcoxon signed rank test with related samples to compare differences between baseline and after follow-up results. Correlations for nonparametric

data were analyzed by the Spearman rank correlation coefficients. Statistical analyses were performed with SPSS V.24 and $p<0.05$ was considered as statistically significant.

Results

Baseline characteristics of the study groups are given in **Table 1**. RA patients were mostly positive for CCPAb and RF and they suffered from active disease as assessed by DAS28. ESR and CRP values were significantly higher in both RA groups compared with the controls (**Table 1**). Periodontal findings, recorded by BOP, PD≥4mm and PIBI, were more frequent in both RA groups compared with the controls. BOP was significantly higher (p=0.032) at baseline in ERA group *vs*. CRA group, but no more at follow-up (p= 0.383). No statistically significant difference with respect to PD≥4mm and PIBI was found at baseline (p=0.511 and p=0.273, respectively) or at follow-up (p=0.115; p=0.143, respectively) between ERA and CRA patients. Salivary MMP-8 concentration and MMP-8/TIMP-1 ratio were significantly higher in ERA group at baseline compared with CRA and control groups, as given in **Table 1**.

Study parameters in ERA group with respect to synthetic DMARDs

RA disease activity decreased significantly in each of the medication groups during the study. Number of teeth decreased significantly in the double therapy group (28 [23-28] to 27 [22-28], p=0.034). CRP decreased significantly from 7 (4-22) to 4 (2-6), (p=0.002). The number of PD≥4mm [6 (3-14) to 12 (5-18), p=0.020] and the median of PIBI [6 (3-14) to 12 (6-19), p= 0.016] increased significantly in the monotherapy group during the study. At follow-up, the MMP-8/TIMP-1 ratio was significantly higher in patients with triple therapy compared with mono- and double therapy groups **(Table 2)**. In other study parameters, there were no significant changes between the baseline and follow-up values within each medication group or between the medication groups when comparing the groups at baseline or after follow-up (**Table 2)**.

Study parameters in CRA group with respect to biological DMARDs

During the follow-up, CRP decreased significantly in patients starting a biological DMARD on the background of monotherapy [6 (2-24) to 2 (2-10), p= 0.028] and DAS28 score diminished significantly in the group with background of DMARD triple therapy [2.9 (2.3-4.2) to 1.7 (1.1-2.1), p= 0.028]. BOP also decreased significantly: 19 (7-25) to 11 (3-19), (p= 0.028). No other significant findings were observed in the study parameters among CRA patients using combinations of synthetic DMARDs combined with biological medication during the study (**Table 3).**

In CRA patients, a significant correlation was found between salivary MMP-8 values and MMP-8/TIMP-1 ratio and PIBI, see **Table 4.** Further, **Table 5** gives the concentrations of salivary MMP-8 and the ratios of salivary MMP-8/TIMP-1 with respect to medians of PIBI in both RA groups at baseline and after follow-up and in controls. The MMP-8/TIMP-1 ratio was significantly higher at baseline in ERA patients with higher PIBI values. In CRA patients, the concentration of MMP-8 and the MMP-8/TIMP-1 ratio were significantly elevated with higher PIBI values at baseline and after follow-up. Also, the concentration of MMP-8 and the MMP-8/TIMP-1 ratio associated with higher PIBI values in controls **(Table 5).**

When comparing the differences of salivary MMP-8 concentration in the CRA patients between the subgroups in relation to the used biologic medication (adalimumab, etanercept, golimumab, certolizumab pegol, anakinra, rituximab), or in relation to the TNF-α inhibitors versus the non-TNF-α inhibitors, no significant differences were observed. Neither did the use of glucocorticoids seem to affect salivary MMP-8 concentration in the RA patients (data not shown).

Discussion

To the best of our knowledge, this is the first report on the impact of DMARDs on salivary MMP-8 values in RA patients in a prospective follow-up scheme. RA patients had poorer periodontal health, assessed by BOP and PIBI, when compared with controls. Significantly elevated BOP and PIBI reflected in higher salivary MMP-8 levels already at the early stage of RA. Compared with CRA patients, BOP was significantly increased in ERA group at baseline, but no other significant differences were observed in periodontal parameters between ERA and CRA groups during the study. Contrary to our expectation, the use of antirheumatic medications (either synthetic or biologic) did not affect salivary MMP-8 values of the RA patients. Although synthetic DMARDs remarkably decreased inflammation in joints during the follow-up, no significant changes were observed in salivary MMP-8 levels after the follow-up of 16 months. This result remained the same also after dividing the patients into subgroups according to mono-, double or triple therapy; hence the synthetic DMARDs did not seem to have any effect in this regard. Thus, the synthetic DMARDs did not seem to disturb the evaluation of periodontal health by the salivary MMP-8 analysis.

Hosts innate, inflammatory and adaptive immune response to the microbial invasion affect to the development of periodontitis with the contribution of behavioral, environmental and genetic factors (Silva et al., 2015). Further, a new model of pathogenesis explains that periodontitis originates by a synergistic and dysbiotic microbial community (polymicrobial synergy and dysbiosis model, PSD model) rather than by a select bacterial complex (Hajishengallis & Lamont, 2012). As the periodontal microbiota change from symbiotic to a dysbiotic stage, abundance of characteristic cytokines and inflammatory mediators are filtered to the periodontal tissue and lead to tissue destruction.

We have recently reported that in this study population, salivary MMP-8 associated with periodontal parameters (Äyräväinen et al., 2018). Here we show that salivary MMP-8 levels were increased already at the beginning of the study in ERA patients probably indicating poor periodontal health, which even got worse in the monotherapy group during the follow-up. This may also reflect the progressive RA associated with active inflammation, revealing the continuous and ongoing inflammatory burden in the joints, which, in fact, may also systematically increase the salivary MMP-8 levels via increased serum concentrations of MMP-8 (Ben-Aryeh et al., 1978).

In the CRA patients, the salivary MMP-8 concentrations correlated with PIBI scores similarly to what was observed in the controls. After the introduction of the biologic DMARDs, the salivary MMP-8 levels increased, even though not significantly. This might be due to ongoing rheumatological inflammation but also because of the patient´s susceptibility to infection due to the biologic medication. This trend was especially clearly observed in those patients who received double and triple therapy together with the biologic DMARDs (TNF-α inhibitor, mostly adalimumab or etanercept). In further analysis, when chronic RA patients were divided into subgroups (adalimumab, etanercept, golimumab, certolizumab pegol, anakinra or rituximab) according to the used biologic medications, to find out whether the medications influenced the association between salivary MMP-8 concentrations and PIBI scores, or if they linked to the number of periodontal pockets, no significant associations were detected. Neither any significant differences were found between the different classes of biological DMARDs when TNF-α inhibitors or non-TNF-α inhibitors had been used.

Recently, a novel active matrix metalloproteinase (aMMP8) point of care/chair-side mouth-rinse test has been demonstrated to quantitatively identify periodontal disease when the patient has at least two deep periodontal pockets (Nwhator et al., 2014; Heikkinen et al., 2016; Sorsa et al., 2017).

It is important to diagnose periodontitis since RA patients often have diminished manual dexterity and thus difficulties in maintaining satisfactory daily oral hygiene; they consequently are liable to periodontitis. This, in turn, may cause systemic low-grade inflammation detrimental to the patients with RA, as earlier discussed.

The strength of the current prospective study was that we have a well-characterized patient population consisting of DMARD-naïve patients with early RA and of patients with chronic active RA whom we have restudied after the introduction of synthetic or biological DMARDs. The weakness, however, was the comparatively small number of patients although we recruited all eligible patients during the set timeline. A lack of detailed information of periodontal treatment received by the patients during the follow-up period was also a limitation of this study. Furthermore, for practical reasons, the control participants could only be examined once. Therefore, the current results could only partly confirm our study hypothesis.

In conclusion, RA patients regardless their disease stage are exposed to inflammation, such as periodontal disease. Antirheumatic medications, synthetic or biologic drugs, seemed to have no significant relevance with respect to periodontal diagnosis. However, more studies with larger patient materials are needed for further conclusion about the effect of DMARDs on saliva/oral fluid biomarkers in RA patients with periodontitis.

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Competing interests

Dr Timo Sorsa is an inventor of US-patents 5652223, 5736341, 5866432 and 6143476.

Other authors declare no competing interests.

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Table 1. Baseline characteristics of the study groups

	Early RA			Chronic RA			Controls	
	Baseline N=53	**Follow-up N=47**	***p*¶**	**Baseline N=28**	**Follow-up N=26**	***p*¶**	**N=43**	***p*¶¶**
RF positive	42 (79.2)			18 (69.2)			3 (8.1)	**<0.001***
CCPAb positive ‡	37 (77.1)			15 (78.9)				0.574*
ESR, mm/h	20 (11–34)	9 (5-16)	**<0.001**	20 (9-46)	16 (7-31)	**0.038**	2 (2-10)	**<0.001**
CRP, mg/l	6 (3-14)	3 (2-6)	**0.001**	18 (5-30)	10 (2-21)	**0.012**	2 (2-3)	**<0.001**
DAS28	4.0 (3.2–4.8)	2.4 (1.7–2.9)	**<0.001**	4.1 (3.0–4.9)	3.1 (2.0–3.9)	**0.003**		0.974
Number of teeth	27 (23–28)	27 (22–28)	**0.024**	27 (22–28)	27 (22–28)	0.317	27 (25–28)	0.628
BOP per cents sites	15 (10–26)	13 (6-21)	0.124	9 (5-19)	8 (3-22)	0.903	4 (2-8)	**<0.001**
PD≥4mm ‡‡	45 (84.9)	43 (81.1)	0.250	25 (89.3)	21 (75.0)	1.000	28 (65.1)	**0.012**
PIBI	10 (3-18)	9 (6-19)	0.907	5 (3-15)	4 (1-16)	0.856	1 (0-3)	**<0.001**
Salivary MMP-8, ng/ml	311.2 (105.6–524.8)	221.0 (128.1–452.8)	0.800	114.8 (40.8–290.8)	175.6 (75.0–391.8)	0.221	113.6 (76.0–226.8)	**0.010**
MMP-8/TIMP-1 ratio	2.5 (0.9-5.5)	1.9 (1.2–4.0)	0.775	1.0 (0.6-2.3)	1.2 (0.7-2.8)	1.000	1.3 (0.8-2.7)	**<0.001**

The results are presented as number of patients (%) or median with interquartile range (IQR).N, number of patients; RA, rheumatoid arthritis; CRA, chronic RA; ERA, early RA; RF, rheumatoid factor; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; CCPAb, anticyclic citrullinated peptide antibody; DAS28, disease activity score (28-joint); BOP, bleeding on probing; PD, pocket depth; PIBI, periodontal inflammatory burden index.
‡ data missing from 5 ERA patients and from 9 CRA patients. ‡‡ at baseline data missing from 3 ERA patients, after follow-up data missing from 7 ERA and from 3 CRA patients.
* *p* value by Chi-Square crosstabulation; ¶ *p* value by nonparametric Wilcoxon test comparing related samples between baseline and follow-up. ¶¶ *p* value by nonparametric Mann-Whitney U-test or Kruskal-Wallis test for other variables comparing study groups at baseline. Statistically significant *p* values are shown in bold.

Table 2. Parameters in ERA patients at baseline and after follow-up with respect to initiation of synthetic DMARDs

Parameter	Monotherapy N=17			Double therapy N=18			Triple therapy N=9				
	baseline	follow-up	*p*¶	baseline	follow-up	*p*¶	baseline	follow-up	*p*¶	*p*¶¶	*p*¶¶¶
DAS28	3.5 (2.6–5.5)	2.4 (1.5–2.9)	**0.003**	4.3 (3.4–5.0)	2.4 (1.8–3.0)	**<0.001**	4.4 (3.0–4.7)	2.7 (1.2–3.3)	**0.043**	0.350	0.947
Duration of symptoms, years	0.4 (0.3-1.1)			0.3(0.3-0.7)			0.4 (0.3-1.9)			0.391	
CRP, mg/ml	5 (2-14)	4 (2-12)	0.688	7 (4-22)	4 (2-6)	**0.002**	3 (3-13)	2 (2-3)	0.090	0.414	0.136
Number of teeth	23 (20–28)	23 (20–28)	1.000	28 (23–28)	27 (22–28)	**0.034**	27 (22–28)	27 (21–28)	0.317	0.594	0.781
BOP	13 (9-35)	14 (6-22)	0.463	16 (8-27)	14 (4-24)	0.649	21 (10–27)	12 (7-14)	0.068	0.956	0.619
PD≥4mm	6 (3-14)	12 (5-18)	**0.020**	11 (4-30)	9 (3-31)	0.697	14 (6-21)	10 (7-16)	0.105	0.407	0.904
PIBI	6 (3-14)	12 (6-19)	**0.016**	11 (4-31)	9 (3-31)	0.568	14 (6-21)	10 (7-16)	0.105	0.407	0.898
Salivary MMP-8, ng/ml	235.2 (96.6–401.2)	268.0 (165.0–475.6)	0.463	166.6 (62.8–355.1)	140.4 (81.0–272.0)	0.469	386.4 (248.2–777.8)	222.8 (175.8–666.2)	0.767	0.141	0.163
MMP-8/TIMP-1 ratio	1.6 (0.7-3.8)	2.5 (1.9–4.7)	0.163	1.0 (0.5-3.3)	1.2 (0.5-2.1)	0.427	4.4 (2.8–6.2)	2.9 (1.5–4.5)	0.260	**0.039**	**0.007**

ERA, early rheumatoid arthritis; DMARDs, disease modifying antirheumatic drugs; DAS28, disease activity score (28 joint); CRP, C reactive protein; N, number; BOP, bleeding on probing; PD, pocket depth; PIBI, periodontal inflammatory burden index; MMP-8, matrix metalloproteinase-8; TIMP-1, tissue inhibitor for MMPs. Results are medians with interquartile range (IQR).

¶ *p* value by nonparametric Wilcoxon test comparing related samples between baseline and follow-up.

¶¶ *p* value by nonparametric Kruskal-Wallis test comparing patients at baseline prior to start of monotherapy, double therapy, or triple therapy with synthetic DMARD(s).

¶¶¶ *p* value by nonparametric Kruskal-Wallis test comparing monotherapy, double therapy and triple therapy groups after follow-up, of a mean of 15.9±6.1 months.

p value significant at the level 0.05. Statistically significant *p* values are shown in bold.

Table 3. Parameters in CRA patients at baseline and after follow-up with respect to initiation of biological DMARDs

Parameter	Monotherapy N= 8			Double therapy N= 11			Triple therapy N=7				
	baseline	follow-up	*p*¶	baseline	follow-up	*p*¶	baseline	follow-up	*p*¶	*p*¶¶	*p*¶¶¶
DAS28	4.6 (2.3–4.9)	2.8 (1.8–4.4)	0.600	4.3 (3.7–5.9)	3.6 (2.9–4.7)	0.051	2.9 (2.3–4.2)	1.7 (1.1–2.1)	**0.028**	0.085	0.068
Duration of symptoms, years	21.4 (11.7–23.9)			7.7 (7.0–18.8)			10.7 (1.8–14.7)			0.236	
CRP, mg/ml	6 (2-24)	2 (2-10)	**0.028**	28 (15–55)	15 (6-32)	0.059	17 (2-29)	7 (2-19)	0.144	0.089	0.084
Number of teeth	26 (21–28)	26 (21–28)	1.000	26 (21–28)	25 (18–28)	1.000	28 (26–28)	28 (26–28)	0.317	0.182	0.302
BOP	19 (7-25)	11 (3-19)	**0.028**	8 (6-15)	5 (2-24)	0.889	5 (3-21)	11 (2-37)	0.138	0.361	0.970
PD≥4mm	5 (2-20)	5 (2-17)	0.399	4 (0.15)	3 (1-14)	0.674	7 (4-15)	6 (1-23)	0.752	0.819	0.848
PIBI	5 (2-21)	5 (2-17)	0.399	4 (0-15)	3 (1-14)	0.674	7 (4-15)	9 (2-35)	0.500	0.825	0.743
Salivary MMP-8, ng/ml	192.2 (42.6–303.1)	184.8 (58.8–375.9)	0.899	110.8 (40.8–456.4)	156.8 (75.0–333.7)	0.721	114.8 (75.2–180.4)	335.8 (164.0–708.0)	0.166	0.558	0.093
MMP-8/TIMP-1 ratio	1.0 (0.6-3.5)	1.0 (0.5-3.2)	0.161	1.5 (0.5-2.3)	1.2 (0.8-2.6)	0.799	0.9 (0.7-1.5)	2.1 (1.0–6.1)	0.345	0.491	0.290

CRA, chronic rheumatoid arthritis; DMARDs, disease modifying antirheumatic drugs; DAS28, disease activity score (28 joint); CRP, C reactive protein; N, number; BOP, bleeding on probing; PD, pocket depth; PIBI, periodontal inflammatory burden index; MMP-8, matrix metalloproteinase-8; TIMP-1 tissue inhibitor for MMPs. Results are medians with interquartile range (IQR).

¶ *p* value by nonparametric Wilcoxon test comparing related samples between baseline and follow-up.

¶¶ *p* value by nonparametric Kruskal-Wallis test comparing patients at baseline grouped according to the ongoing mono therapy, double therapy or triple therapy with synthetic DMARDs.

¶¶¶ *p* value by nonparametric Kruskal-Wallis test comparing patients according to the background use of synthetic DMARDS as monotherapy, double therapy or triple therapy after follow-on biological DMARDs with a mean of 15.9±6.1 months.

p value significant at the level 0.05. Statistically significant *p* values are shown in bold.

Table 4. Correlation of salivary MMP-8 concentration and MMP-8/TIMP-1 ratio with periodontal inflammatory burden

	ERA			CRA			Controls
	PIBI at baseline R_s ***p*¶**	**PIBI after follow-up** R_s ***p*¶**	**correlation for the change** R_s ***p*¶**	**PIBI at baseline** R_s ***p*¶**	**PIBI after follow-up** R_s ***p*¶**	**correlation for the change** R_s ***p*¶**	**PIBI** R_s ***p*¶**
MMP-8, ng/ml	0.298 **0.035**	0.185 0.229	0.002 0.990	0.720 **<0.001**	0.585 **0.002**	0.138 0.529	0.391 **0.010**
MMP-8/TIMP-1 ratio	0.301 **0.034**	0.220 0.156	0.043 0.787	0.685 **<0.001**	0.610 **0.002**	– 0.068 0.756	0.391 **0.010**

MMP-8, matrix metalloproteinase-8; TIMP-1, tissue inhibitor for MMPs; PIBI, periodontal inflammatory burden; ERA, early rheumatoid arthritis; CRA, chronic rheumatoid arthritis

¶correlation R_s Spearman correlation, 2-tailed *p* value significant at the 0.05 level

Statistically significant *p* values are shown in bold.

Table 5. Salivary MMP-8 concentration and MMP-8/TIMP-1 ratio compared to periodontal inflammatory burden in the study groups during the study

	ERA			CRA			Controls				
	PIBI ≤ median	**PIBI >median**	***p*¶**	**PIBI ≤ median**	**PIBI >median**	***p*¶**	**PIBI ≤ median**	**PIBI >median**	***p*¶**	***p*¶¶**	***p*¶¶¶**
MMP-8 baseline	185.2 (101.6–385.2)	356.0 (189.6–718.0)	0.124	54.0 (35.0–138.2)	204.8 (109.7–636.3)	**0.008**	102.8 (64.0–172.0)	151.4 (108.8–538.5)	**0.008**	**0.003**	0.228
MMP-8 follow-up	216.4 (103.6–584.0)	252.0 (172.6–390.0)	0.404	82.4 (41.0–159.6)	407.6 (177.3–636.2)	**0.001**				**0.008**	0.246
MMP-8 change	16.8 (-85.6–159.0)	8.6 (-138.9–118.1)	0.417	– 1.6 (-48.0–76.8)	20.2 (-30.4–207.7)	0.321				0.701	0.250
MMP-8/TIMP-1 baseline	1.5 (0.8-3.1)	3.5 (1.6–6.0)	**0.037**	0.6 (0.4-1.4)	1.5 (0.9-3.8)	**0.014**	0.9 (0.5-1.7)	2.2 (0.9-3.8)	**0.009**	**0.023**	0.165
MMP-8/TIMP-1 follow-up	1.6 (0.9-3.6)	2.4 (1.3–4.2)	0.198	0.8 (0.5-1.2)	2.8 (1.0–4.6)	**0.004**				**0.031**	0.793
MMP-8/TIMP-1 change	0.1 (-1.3–1.4)	0.1 (-1.3–0.7)	0.722	– 0.1 (-0.8-0.3)	– 0.1 (-0.9-1.6)	0.780				0.600	1.000

MMP-8, matrix metalloproteinase-8; TIMP-1, tissue inhibitor for MMPs; PIBI, periodontal inflammatory burden; RA rheumatoid arthritis
PIBI median in ERA at baseline 10, after follow-up 9; for the change from baseline to follow-up 0; in CRA at baseline 5, after follow-up 4;for the change from baseline to follow-up 0; controls 1. Results are medians with interquartile range (IQR).
¶*p* value by nonparametric Mann-Whitney U-test comparing differences between ≤median and > median in each study group
¶¶*p* value by nonparametric Kruskal-Wallis test comparing differences between Era, Cra and control groups when PIBI≤median
¶¶¶*p* value by nonparametric Kruskal-Wallis test comparing differences between Era, Cra and control groups when PIBI>median
Statistically significant *p* values are shown in bold.